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TITLE : DEGRADATION PROMOTER OF DESMOSOME

ABSTRACT : PROBLEM TO BE SOLVED: To obtain degradation promoter of desmosome, useful as a preparation for maintaining a state of healthy skin by using a specific dicarboxylic acid derivative as an active ingredient.

SOLUTION: This degradation promoter of desmosome or a skin preparation for external use is obtained by formulating 0.01-20wt.% dicarboxylic acid derivative of the formula, HOOC-L-COON [L is a single bond or a (substituted) 1-8C alkylene chain], (e.g. oxalic acid and malonic acid) based on the amount of the objective preparation. The physiological peeling off of a horny substance is improved because trypsin-like enzyme activities and/or chymotrypsin-like enzyme activities concerning decomposition of a protein in the horny substance is enhanced.

(a)

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DEGRADATION PROMOTER FOR DESMOSOME

Abstract:

[Object] To provide a preparation for keeping a healthy state of the skin.

[Means Therefor] An agent for external application to the skin, particularly, a degradation promoter for desmosome, containing a dicarboxylic acid of the formula HOOC-L-COOH as an effective ingredient (in the formula, L is a simple linkage or, in some cases, it is an alkylene group having 1 to 6 carbon(s) which may be substituted with an alkyl group, a hydroxyl group, an ester group, an amino group, an amide group, an oxo group or a carboxyl group).

What is Claimed is:

1. A degradation promoter for desmosome which is characterised in containing at least one dicarboxylic acid derivative represented by the following formula (I) or a salt thereof as an effective ingredient.

$$HOOC\text{-}L\text{-}COOH \qquad (I)$$

[in the formula, L is a simple linkage or an unsubstituted or substituted alkylene chain having 1 to 8 carbon(s) where the substituent in the substituted case is a group which is selected from the group consisting of an alkyl group, a hydroxyl group, an amino group, a saturated or unsaturated amide residue (R^1CONH-), an oxo group (=O), a carboxyl group and a saturated or unsaturated aliphatic oxycarbonyl residue (R^2OCO-) and one or more substituent(s) as such may be present depending upon the carbon numbers of the above alkylene group or each two hydrogen atoms on adjacent carbon atoms of the alkylene group may be detached to form a simple linkage].

2. The degradation promoter according to claim 1, wherein degradation of desmosome is caused by the protein-degrading activity *per se* existing in horny layers.

3. A degradation promoter for desmosome which is characterised in containing at least one dicarboxylic acid derivative represented by the following formula (II) or a salt thereof as an effective ingredient.

$$HOOC\text{-}L'\text{-}COOH \qquad (II)$$

[in the formula, L' is a simple linkage or an unsubstituted or substituted alkylene chain having 1 to 8 carbon(s) where the substituent in the substituted case is a group which is selected from the group consisting of an alkyl group, a hydroxyl group, an amino group, a saturated or unsaturated amide residue (R^1CONH-), an oxo group (=O), a carboxyl group and a saturated or unsaturated aliphatic oxycarbonyl residue (R^2OCO-) and one or more substituent(s) as such may be present depending upon the carbon numbers of the above alkylene group or each two hydrogen atoms on adjacent carbon atoms of the alkylene group may be detached to form a simple linkage with a proviso that, when there is a hydroxyl group as the substituent, there is also an amino group or the above-mentioned amide

residue as another substituent at the same time].

Detailed Description of the Invention:

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to an agent for external application to the skin or a degradation promoter for desmosome of the skin where an aliphatic dicarboxylic acid derivative is contained as an effective ingredient. The present invention is mainly utilized in the technical field of cosmetics.

[0002]

[Prior Art]

It has been made already clear from the studies up to now that a moisturizer effectively acts on the skin which is made dry whereby the so-called dry skin is generated (refer, for example, to Tatsuya Ozawa, et al.: "Role of Moisturizers in Moisturization of the Skin", *Hifu*, 27, 276-288 (1985)). Although there has been no specific explanation for the mechanism of the moisturizer for the improvement of desquamation which is a characteristic of dry skin, it has been confirmed that, at present, various kinds of moisturizers have been used for agents for external application for the skin and that certain effects have been achieved as well.

[0003] With regard to the representative examples of such moisturizers or skin softeners, lactic acid which is classified under an α-hydroxycarboxylic acid has been widely used. It has been also known that cosmetics particularly having a softening action to horny layer are compounded with the above lactic acid being classified under α-hydroxycarboxylic acid as well as with an α-hydroxycarboxylic acid having an alkyl group which is longer than methyl group or with that having a carboxyl group as a substituent such as citric acid or tartaric acid (Japanese Laid-Open Patent No.58/8007). It has been further known to use 2-(or α-)hydroxycarboxylic acid or a related compound thereto for a composition which mitigates the dermatoscientific aging characteristics (Japanese Laid-Open Patent No. 05/139947). It has been still further known a composition for the treatment of symptom of xeroderma containing a specific hydroxycarboxylic acid, ketocarboxylic acid

or ester thereof and a lipid component such as ceramide (Japanese Laid-Open Patent No. 06/157283).

[0004] The Japanese Laid-Open Patent No. 58/8007 suggests that an α-hydroxycarboxylic acid improves elasticity of horny layer pieces and accordingly that it achieves a softening effect to the skin. The Japanese Laid-Open Patent No. 05/139947 suggests that a 2-(or α-)hydroxycarboxylic acid has an action of decreasing the horny cell aggregation in the horny layer while it is ineffective in the outer layer of the horny layer. The Japanese Laid-Open Patent No. 06/157283 suggests that the said composition visually improved the xeroderma in an *in vivo* test.

[0005] Incidentally, it has a common theory that lipid participates in adhesion of horny layer (or layered structure of horny cells) but, recently, there has been a suggestion on the basis of finding by means of an electron microscope that desmosome is an essential structure for adhesion of horny cells (refer, for example, to Kitajima, *Koyu Kaishi*, Vol. 15, No. 4 (1991), pages 225-230). Kitajima also proposes a presumption in the said article that digestion of desmosome by protease is the first element for exfoliation of horny layers. In addition, A. Lundstrobe, et al. suggest in *Acta Derm. Venereol.* (Stockh), 1991: 71: 471-474 that, with regard to desquamation under an *in vivo* condition, chymotrypsin-like enzyme having a molecular weight of 25 kDa which is believed to be present in horny layer plays a certain role.

[0006] In the meanwhile, the present inventors have investigated of desquamation of horny layers and made clear that, in addition to the above-mentioned trypsin-like enzyme, there is another trypsin-like enzyme having a molecular weight of about 30 kDa as an intrinsic protease having a possibility of participating in desquamation (*Arch. Dermatol. Res.* (1994) 286: 249-253). It has been further made clear that a moisturizer arranges the place (i.e., aqueous environment) necessary for expression of the above-mentioned two kinds of enzymatic activities in the skin, particularly in desmosome, whereby the healthy skin is able to be maintained (refer, for example, to *Fragrance Journal* (1995), 13-18).

[0007]

[Problems that the Invention is to Solve]

It is necessary that, in order to keep the healthy and beautiful appearance of not only the skin of patients suffering from xeroderma as mentioned above but also and particularly the skin of healthy people, there is a good balance between the formation of horny layer constituted from horny cells which are dead cells of epidermal cells and detachment thereof due to a physiological exfoliation.

[0008] It is of course true that a moisturizer participates in desquamation of horny layers and plays a role in keeping healthy skin. However, in the case of an individual where the activities of above-mentioned two enzymes lower, a sufficient exfoliation of horny layers will not be achieved only by arranging the environment for expression of such activities by a moisturizer.

[0009] Accordingly, an object of the present invention is to provide a preparation not only for a purpose of moisturization but also for a purpose of enhancement of the activities *per se* of at least the above-mentioned two enzyme.

[0010]

[Means for Solving the Problems]

The present inventors have found that desquamation of horny layer was resulted via degradation of protein, at least desmoglein, in horny layers by the above-mentioned enzyme. The mechanism of desquamation of horny layers as such is quite contrary to the fact that the compounds mentioned in the above-mentioned Japanese Laid-Open Patent No. 05/139,947 are not dependent upon the action in the outer layer of horny layers and is entirely new.

[0011] The present inventors have further found that degradation of desmosome is promoted by an aliphatic dicarboxylic acid which does not always carries a liberated hydroxyl group and that, for such an action, it is necessary that at least two carboxyl groups have a certain special distance.

[0012] Thus, in accordance with the present invention, there is provided a degradation promoter for desmosome which is characterised in containing at least one dicarboxylic acid derivative represented by the following formula (I) or a salt thereof as an effective ingredient.

HOOC-L-COOH (I)

[in the formula, L is a simple linkage or an unsubstituted or substituted alkylene chain having 1 to 8 carbon(s) where the substituent in the substituted case is a group which is selected from the group consisting of an alkyl group, a hydroxyl group, an amino group, a saturated or unsaturated amide residue (R^1CONH-), an oxo group (=O), a carboxyl group and a saturated or unsaturated aliphatic oxycarbonyl residue (R^2OCO-) and one or more substituent(s) as such may be present depending upon the carbon numbers of the above alkylene group or each two hydrogen atoms on adjacent carbon atoms of the alkylene group may be detached to form a simple linkage].

[0013] Among the above-mentioned compound, certain dicarboxylic acid derivatives except the compound where L has only a free hydroxyl group as a substituent have not been mentioned in prior art documents for their use as an agent for external application to the skin. Therefore, in accordance with the present invention, there is also provided an agent for external application to the skin containing such a derivative as an effective ingredient and having a broad use including the promotion of degradation of desmosome as mentioned above.

[0014]

[Specific Embodiments of the Invention]

The degradation promoter of desmosome according to the present invention is able to not only give an aqueous environment suitable for desquamation of horny layer to the skin but also enhance the degrading ability *per se* to proteins (at least desmoglein and, in some cases, desmocollin as well) existing in the horny layers. Such an action means that a candidate compound or component is able to enhance the above-mentioned trypsin-like enzymatic activity and/or chymotrypsin-like enzymatic activity under an appropriate water-containing environment. Such an enzymatic activity is able to be evaluated by measuring the degree how much the residual rate of desmoglein in desmosome can be reduced by the candidate substance.

[0015] Therefore, the compound in accordance with the present invention which will be mentioned in detail as hereunder is able to statistically significantly reduce the residual rate of the above-mentioned desmoglein.

With regard to the compound as such, the compound represented by the formula (I) is exemplified and the said compound may, if necessary, be used as an inorganic salt with alkaline metal such as sodium or lithium, alkaline earth metal such as calcium or magnesium or ammonium or as a physiologically acceptable salt with an organic amine.

[0016] More specifically, L in the formula (I) is selected from the following groups.

[0017] (A) A simple linkage or an unsubstituted alkylene chain having 1 to 8 or, preferably, 1 to 6 carbon(s) such as methylene, ethylene and propylene.

[0018] Examples of the compound of the formula (I) specified by the above definition are oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid and octanedioic acid.

[0019] (B) Substituent when L is substituted.

(i) Examples of the alkyl group are straight or branched alkyls having 1 to 22 carbon(s) such as methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, octyl, decyl, eicosyl and docosyl. Theoretically, up to 16 alkyl groups are able to be bonded to an alkylene chain depending upon the carbon numbers of an alkylene chain bonding to an alkyl and, in the case of a lower alkyl group having 1 to 6 carbon(s), it is preferred that up to 2 is/are bonded and, in the case of medium or higher alkyl group, it is preferred that one is bonded. In addition, an alkyl group is able to be a substituent for an alkylene chain (may be called an L chain as well) together with one or more substituent(s) which will be mentioned later.

[0020] (ii) Hydroxyl group

At least one hydroxyl group may be bonded to each one carbon atom of the alkylene chain (i.e. no acetal is formed) and, therefore, up to 8 hydroxyl groups may be bonded to an alkylene chain. Examples of the hydroxyl-substituted L chain are -CHOH-, $-CH_2CH(OH)-$, $-CH(OH)CH(OH)-$, $-CH_2CH(OH)CH_2-$, $-CH(OH)CH_2CH_2-$, $-CH(OH)CH_2(OH)-$, $-CH(OH)CH(OH)CH(OH)-$, $-CH(OH)CH_2CH_2CH_2-$,

[0021] $-[-CH(OH)-]_4-$, $-[-CH(OH)-]_5-$ and $-[-CH(OH)-]_6-$. The compounds having the above L-chains successively correspond to tartronic acid, malic

acid and tartaric acid and other compounds are also able to be prepared as an oxide of saccharide for example. In the above-mentioned hydroxyl-substituted L-chain, the above alkyl group may be bonded to each carbon atom.

[0023] (iii) A saturated or unsaturated fatty acid ester residue (R^1COO-) is a residue derived from a natural or synthetic fatty acid having 2 to 26 carbons or, to be more specific, a group which is formed from the above hydroxyl-substituted L-chain and the above fatty acid. Accordingly, the residue may be present in plural depending upon the numbers of hydroxyl group on the L-chain; preferably there are present two or, particularly, there is present one.

[0024] The fatty acid which forms an ester residue as mentioned above is preferably that which is derived from natural lipid and is a saturated fatty acid derived from rice bran oil wax, lanoline, coconut oil, palm oil, milk fat, etc. (such as cerotic acid, lignoceric, behenic acid, arachidic acid, stearic acid, palmitic acid, myristic acid, lauric acid, capric acid, caprylic acid, carproic acid and butyric acid) or an unsaturated acid derived from land animal fat (e.g., tallow), marine animal oil (particularly fish liver oil) and other plant oil (e.g., coriander oil and evening primrose seed oil) (such as palmitoleic acid, petroceric acid, oleic acid, elaidic acid, linoleic acid, γ-linolenic acid and arachidonic acid). The above fatty acid may also have hydroxyl group such as in the case of ricinoleic acid and α-hydroxylinoleic acid.

[0025] As mentioned above, such an ester residue may be present in L-chain together with one or more hydroxyl group(s) and alkyl group(s) depending upon the carbon numbers of the L-chain.

[0026] (iv) The amino group ($-NH_2$) may be substituted with one or two lower alkyl group(s) in addition to the amide residue which will be mentioned later although unsubstituted one is preferred. The amino group may also be present in plural on the L-chain depending upon the carbon numbers of the L-chain although it is preferred that only one is present. It may also be preferred on the L-chain together with at least one of the above substituents (i) to (iii) and, preferably, it is present together with a hydroxyl group or a saturated or unsubstituted fatty acid ester residue. As a result of the presence of such substituents on the L-chain, representative examples of the compound of the formula (I) are aspartic acid and glutamic acid.

[0027] (v) The saturated or unsaturated fatty acid amide residue is formed from a fatty acid which forms the ester residue mentioned in the above (iii) and an amino group mentioned in the above (iv). It is also possible that, as same as in the case of the amino group of (iv), the said residue may be present on the L-chain together with the substituents of (i) to (iii).

[0028] (vi) Typical examples of the compound of the formula (I) having an oxy group (=O) as a substituent are oxoglutaric acid ($HOOCCH_2CH_2C(=O)COOH$) and oxiadipic acid. Although an oxo group may be usually present on the L-chain together with the alkyl group of (i), it is better to be present solely.

[0029] (vii) Carboxyl group may also be present in plural corresponding to the carbon numbers of the L-chain or may also be present together with at least one substituent from (i) to (v). Typical examples of a compound of the formula (I) having such a substituent are citric acid or an ester compound at its hydroxyl group and isocitric acid or an ester compound at its hydroxyl group.

[0030] (viii) The saturated or unsaturated aliphatic oxycarbonyl residue (R^2COO-) is particularly preferably a group which is formed from an alcohol in natural fat/oil or wax and a carboxyl group mentioned in the above (vii). Specific examples of the alcohol which forms such a group are straight-chain alcohols such as octanol, decanol, cetyl alcohol, stearyl alcohol, hexacosanol and octacosanol; branched alcohols such as 14-methylhexadecan-1-ol, 16-methyloctadecanol and 24-methylhexacocosanol; and unsaturated alcohols such as dodecenol and oleyl alcohol. Such a substituent may also be present on the L-chain together with the substituent of the above (i) to (v) and (vii).

[0031] (ix) The L-chain alkylene group may be a simple linkage where each two hydrogen atoms on the adjacent several carbon atoms are detached. Specific examples of such an L-chain are -CH=CH-, -CH=CH-CH_2- and -CH=CH-CH=CH-. Examples of typical examples of the compound (I) having such an L-chain are maleic acid and fumaric acid.

[0032] The compound of the L-chain having the above-mentioned substituents may have two or more stereoisomers and, in the present invention, it may be a mixture of stereoisomers (racemic or diastereomer). The L-chain of the formula (I) and the L'-chain of the formula (II) are the

same except that the latter has no free hydroxyl group.

[0033] The compound of the formula (I) which is specified as above may be made into a preparation by compounding with diluent, adjuvant and other active agent which are used in the technical field of drugs and cosmetics within such an extent that such a thing meets the object of the present invention. Examples of the typical diluent, adjuvant, etc. are moisturizer, surface-active agent, alcohol, water, buffer, chelating agent and skin activator although they are non-limitative.

[0034] The compound represented by the formula (I) or the formula (II) in the degradation promoter for desmosome or the agent for external application to the skin in accordance with the present invention may be made 0.01 to 20% by weight depending upon physical properties of the compound. The application is mostly by way of a direct application to the skin and the dose of the compound of the formula (I) or the formula (II) may be appropriately adjusted depending upon age or skin symptom of the person to be treated.

[0035] When the preparation of the present invention is used as such, morbid thickening of the skin can be prevented and treated and, moreover, in healthy persons, formation of horny layers and detachment thereof by physiological exfoliation can be harmonized.

[0036]

[Examples] As hereunder, the present invention will be illustrated in more detail by way of specific examples.

[0037] Method of evaluation: Residual rate of desmoglein in horny layer sheet

In this method, degree of degradation of desmoglein in horny layer sheet by a trypsin-like enzyme and a chymotrypsin-like enzyme or, in other words, degree of exfoliation of horny layer is evaluated. The lower the residual rate of desmoglein, the higher the promotion of the above enzymatic activity.

[0038] Horny layer in a sheet form collected from healthy person was dipped for 30 seconds in an antiseptic/antifungal solution (60 μl/ml of kanamycin; 0.5% of NaN_3). A 5% aqueous solution (5 μl) of the compound as shown in

the following Table I was applied on both surfaces of 2 mg of the horny layer taken out therefrom. That which was applied with a solution containing no compound was used as a control and the following operation was also carried out for a sample to which the above solution was not applied.

[0039] The horny layer prepared as above was allowed to stand at 37°C for one week under the condition of 30% or less horny layer moisture where degradation of desmosome is insufficient. After that, each horny layer was extracted at 37°C for 15 hours with 0.5 ml of 0.1M Tris buffer (pH 9) containing 9M of urea, 2% of SDS and 1% of mercaptoethanol. Then 0.7 ml of a sample buffer for SDS-PAGE (a Laemii solution in a double concentration) was added to the extract followed by heating for 15 minutes. Each 10 μ of those solutions were taken out and subjected to electrophoresis using a gel of 7.5% concentration. After the electrophoresis, it was transcribed to PVDF, an immunostaining was carried out using an anti-desmoglein antibody and amount of protein was determined. The result is shown in the following Table I as mean values of three runs.

[0040]

Table I

Compound Tested	Residue Rate of Desmoglein(%)
Control	100.00
Nothing applied	88.00
Oxalic acid	10.80
Malonic acid	10.80
Succinic acid	43.50
Glutaric acid	43.50
Adipic acid	94.00
Pimelic acid	75.50
Suberic acid	72.50
Fumaric acid	52.73
Tartronic acid	0.00
Malic acid	24.20
Tartaric acid	24.20
Diacetyltartaric acid	51.28
Citric acid	33.00
Methylmalonic acid	69.34
Methylsuccinic acid	58.13
Dimethylsuccinic acid	47.47

Pentatricarboxylic acid	40.00
Propanetricarboxylic acid	33.00
Glucaric acid	86.60
Aspartic acid	45.00
Glutamic acid	60.00
Sodium glutamate	88.50
N-Acetylglutamic acid	67.15

Example of Preparation

(Components)	(Weight (%))
1,3-Butylene glycol	6.0
Glycerol	4.0
Oleyl alcohol	0.1
POE(20) sorbitan monolaurate	0.5
POE(15) lauryl alcohol ether	0.5
Ethanol	10.0
Aspartic acid	10.0
Pure water	68.9

Preparation

1,3-Butylene glycol and glycerol were dissolved at room temperature in pure water to give an aqueous phase. Other components were dissolved in ethanol and mixed with the above-prepared aqueous phase to dissolve. After that, the mixture was filtered and charged to give a cosmetic lotion.

[0041]

[Advantage of the Invention]

In the present invention, there is provided an agent for external use for the skin which is particularly effective in promoting the physiological exfoliation of horny layer. The meritorious effect as such is in accordance with the present invention where a specific dicarboxylic acid derivative promotes the trypsin-like enzymatic activity and/or chymotrypsin-like enzymatic activity participating in degradation of protein in horny layer. Thus, as a result of the present invention, there is provided an agent for external application to the skin containing a compound belonging to a new category as an effective ingredient where the said compound is able to participate in new detachment of horny layer.

[End]